Int.J.Curr.Microbiol.App.Sci (2020) *9*(6): 1320-1329

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 6 (2020)**
Journal homepage: http://www.ijcmas.com

Review Article https://doi.org/10.20546/ijcmas.2020.906.164

Induction of Mutation in Flower Crops-A Review

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Keywords

Mutation, Gamma ray, EMS, DES and Ornamental plants

Article Info

Accepted:
18 May 2020
Available Online:
10 June 2020

A B S T R A C T

Mutation is the sudden heritable change that occurred in an organism. It may be caused by spontaneous or through artificial induction and the resulted mutant shows change in the gene or chromosomes. Induced mutagenesis techniques have successfully produced and commercialized quite a large number of new promising varieties in different crops worldwide, including ornamental plants. Induced mutagenesis has been most successful in ornamental crops. The improvement achieved through mutation breeding in ornamental crops includes compact growth, attractive variegated leaves and novel flower colour and shapes. Ornamental plants with a rich variety of flower colors and shapes are highly prized, and the production of mutant cultivars that differ in these traits is in demand because all other growth habits are identical. Many species of ornamental plants, such as chrysanthemum (*Chrysanthemum* spp.), rose (*Rosa* spp.), and carnation (*Dianthus caryophyllus*), are vegetatively propagated, making it relatively easy to propagate mutants. This review not only provides examples of successful mutation breeding results using physical and chemical mutagens, but it also describes research on mutagenesis and compares results of gamma ray, EMS and DES using ornamental plants.

Introduction

Mutation is the sudden heritable change that occurred in an organism. It may be caused by spontaneous or through artificial induction and the resulted mutant shows change in the gene or chromosomes (De and Bhattacharjee, 2011). Induced mutagenesis techniques have successfully produced and commercialized quite a large number of new promising varieties in different crops worldwide, including ornamental plants. Both physical and chemical mutagens were used for improving the desired characters of flower and ornamental crops such as amaryllis, asiatic hybrid lily, bougainvillea, chrysanthemum, dahlia, gladiolus, hibiscus, *Lantana depressa* Naud, marigold, rose, tuberose, gerbera, narcissus *etc.* Induced mutations in ornamentals comprise traits such

as altered flower characters (colour, size, morphology, fragrance), leaf characters (form, size, pigmentation), growth habit (compact, climbing, branching) and physiological traits such as changes in photoperiodic response, early flowering, free flowering, keeping quality and tolerance to biotic and abiotic stresses. Mutation breeding holds the key advantage of ability to change one or a few characters of an otherwise outstanding variety without altering the unique part of the genotype. (Datta, 2014).

In any mutation breeding programme, selection of an effective and efficient mutagen agent that induce sudden heritable change is very essential to produce high frequency desirable mutants. Several factors such as properties of mutagens and pH, duration of treatment, temperature *etc.* play pivotal role to produce a desirable mutant. Mutation is induced by physical and chemical mutagen treatment in both seed and vegetative propagated crops. The mechanism of mutation induction is breaking the nuclear DNA and during the process of DNA repair mechanism, new mutations may occur randomly and are heritable.

It is a simple, efficient, rapid and cheap option for obtaining desired genotypes in crops. Induced mutation is one of the most widely used techniques for creating additional variability in desirable character by physical and chemical mutagens. In physical mutagens, atoms are the principal source material. Unstable atoms of same element having different weights giving energy or particles are called radioisotopes and electromagnetic waves associated with nuclear decay are called as radiation and the treatment of an organism or plant with radiation is known as irradiation. It is classified into two groups ionizing and non ionizing radiations. Alpha rays (α), Beta rays (β), X-rays, Gamma ray (γ) and Neutrons belongs to the group of ionizing radiation. Non ionizing radiation includes UV rays only. Ionizing radiations normally causes chromosomal rearrangements and deletions, that results in mutation.

Gamma rays are electromagnetic radiations having shorter wavelength than X rays with more energy and penetrating power. It is produced by a number of isotopes viz., ^{14}C, ^{60}Co, ^{137}Cs etc. for chronic treatments requiring slow irradiation over long periods (De and Bhattacharjee, 2011). Mutation can also be induced chemically with alkylating agents such as Diethyl sulphate (DES) and Ethyl methane sulphonate (EMS). The alkyl group of chemical mutagens reacts with DNA which may change the nucleotide sequence and cause a point mutation. (Broertijes and Harten, 1988). EMS alkylates are guanine bases and leads to mispairing-alkylated G pairs with T instead of C, resulting in primarily G/C to A/T transitions (Bhat *et al.*, 2007). Kayalvizhi *et al.,* (2018) revealed that, tuberose variety Prajwal bulbs treated with different dose of 0.5, 1.0, 1.5, 2.0 and 2.5 kR gamma rays, induced higher proportion of chlorophyll mutants (xantha', 'chlorina' and 'striata'), broad leaved, non-flowering and floral mutants.

Induced mutagenesis in crop improvement

Induced mutation is one of the tools used to create wide variability. Since spontaneous mutations occur at a very low frequency and often do not include the full range of variability, mutations are induced in high frequencies by physical or chemical mutagens (Konzak *et al.*, 1961). Zhao (2002) clearly explained` that mutation induction techniques can greatly increase the gene mutation frequency which helps to develop new variety and also to meet the breeding targets in a relatively shorter period. Several pioneer workers *viz.,* Muller (1927) and Stadler

(1928), Swaminathan (1969) and Datta (2014) demonstrated the potential significance of inducing useful mutation. Physical mutagens commonly used are X-rays, gamma rays, fast moving neutrons, thermal neutrons, radio isotopes and ultraviolet rays. The relative frequency of mutations and chromosomal aberrations can be identified by using different mutagens *viz.*, gamma rays, X-rays, neutrons, etc. The choice of mutagen to be made mostly depends upon the breeding material to be used and the objectives of mutation studies. Gamma irradiation has certain advantages over the chemical mutagens viz., no residual effect; uniformity in penetration; less time consuming and large number of samples can be treated in less time.

It provided on high number of useful mutants and is still showing an elevated potential for improving vegetatively propagated plants. Induction of mutation is an important pathway for the production of new genotypes in asexually propagated species and to enhance natural genetic resources (Jyothi and Singh 2016). Ahloowalia and Maluzynski (2001) reported the use of ionizing radiations like X- rays, gamma rays, neutrons and chemical mutagens for inducing variation in arabidopsis, petunia and antirrhinum.

Zhao (2002) stated that gamma rays was the most commonly used mutagen to induce mutations in fruit trees, ornamental plants, medicinal and aromatic plants, in the past decades of radiation breeding practice in China. Similarly, Wang *et al.,* (2006) also opined that gamma rays were effective in creating mutants and have been successfully used in generation of new crop varieties. Bulbs of tuberose variety Prajwal were subjected to treatments at different doses of gamma ray (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 kR. Leaf abnormalities (leaf texture and chlorophyll variation) were noticed in 1kR and 1.5kR treated bulbs. Gamma rays 0.5 kR resulted in economic traits namely, number of spikes per plant (3 nos.) and number of florets per spike (55 nos.) as reported by Kayalvizhi *et al.*, (2016a).

Induction of mutants by using physical mutagens

The suitable radiation dose for induction of somatic mutations in chrysanthemum has been done by many workers. The dose of gamma rays to be used obviously depends upon the dose of mutagens, plant part and the stage of development. The dose is varied based on the crop, the method of propagation, the number can be handled and the selection method (Broertjes and Van Harten, 1988).

The wide range of sensitivity among different plant species to X-ray or gamma irradiation is well documented (Sparrow *et al.,* 1963). Differences in radio sensitivity among the species are at least 100 fold and over 50 fold was reported within a species irradiated at different stages (Sparrow *et al.,* 1968).

Radiation injury expresses itself after a few weeks or in some cases years as abnormal shape or appearance, reduced growth or yield, loss of reproductive capacity, sometimes wilting and finally death of plants at the higher rate of exposure. The radio sensitivity of plant species was mainly dependant on the nuclear volume (more sensitivity if the DNA content was greater) and ploidy level (less sensitivity if ploidy level was higher). The survival end points which have been found to be most useful in describing radiation effects on plants were LD_{10}, LD_{50}, LD_{90} and LD_{100}; the exposures required to reduce the plant survival by 10, 50, 90 and 100 per cent respectively.

Datta (2014) had given the LD50 value of gamma rays to various flower crops and is presented in table 1.

El-Kholy and Hassan (1983) reported reduced germination in *Sterlitzia regina* by treating the seeds at 500 or 1000 rads of gamma rays either dry or soaked in water for 72 hours. Irradiation reduced average germination from 18 per cent in the control to 9.5 to 14.8 per cent in the treated seeds. Irradiation reduced the number of days for emergence also from 48 in the control to 36 – 47.5 with dry seeds with 500 rads. Talukdar *et al.,* (1997) treated chrysanthemum cultivars *viz.,* Snow Ball, Temptation, Raju, Eva Turner and Grape Bowl with 0.5, 1.5 and 2.5 kR gamma rays. A marked reduction in agronomic characters was observed. Moderate to higher doses of gamma rays (1.5 to 2.5 kR) appeared to be useful in creating stable mutants. Balakrishnan (1997) reported that the LD_{50} for chrysanthemum cvs. CO 1 and CO 2 were 1.0 and 1.5 kR respectively. Mandal *et al.,* (2000) reported the existence of a negative correlation between the dosage level and the survival rates in two chrysanthemum cultivars Poornima and Colchi Bahar.

Banerji and Datta (2002) treated rooted cuttings of *Chrysanthemum morifolium* with 100, 150, 200 and 250 Gy of ^{60}Co gamma rays. Reduction in survival, plant height, no. of branches, leaves, flowers and size were noticed along with increase in foliage and floral abnormalities and chromosomal aberration and delay in flowering at different doses. Srivastava and Mishra (2005) irradiated *Hibiscus rosa-sinensis* cuttings with gamma rays at various doses to induce mutation and observed that 10 Gy dose resulted in a mutant producing significantly smaller sized flowers due to reduced petal length (3.3 cm against 5.2 cm in the control). Puripunyavanich and Boonsirichai (2008) reported that the bulblets of 'Jongkolnee' water-lily irradiated with gamma rays at 0, 50, 100, 150 and 200 grays (20 bulblets/treatment) yielded two mutant lines. One with the white petals but the flower had the irregular shape (150 Gy). The other had purple flowers with normal flower morphology (100 Gy). In Bougainvillea, promising and beautiful variegated mutants were induced by gamma radiation. Stem cuttings of multi-bracted cultivars viz., Cherry Blossom, Banas Beautya, Mahara, Roseville's Delight were treated with 0, 5, 10 and 15 Gy. Chlorophyll variegation in leaves were detected in all four cultivars while somatic mutation in bract colour were detected in chimera forms in the three varieties namely, Cherry Blossom, Banas Beautya and Roseville's Delight. The mutants were isolated in pure form by chimera management and released as varieties namely 'Arjuna', 'Pallavi', 'Mahara Variegata', 'Los Banas Beautya Variegata', 'Los Banas Beautya Variegata Jayanthi' (Banerji, 2008).

In Taiwan, 37 induced mutant varieties were produced in canna by treating rhizomes and young shoots of eleven cultivars with acute (15-30 Gy) and chronic (65-110 Gy) gamma irradiation (Jompuk *et al.,* 2008). Patil and Patil, (2009) suggested to use gamma rays at lower concentrations (Rose- 5 to 7.5 kR, Chrysanthemum- 1.5 to 2 kR, Gladiolus- 1 to 5 kR, Tuberose- 0.5 to 1 kR, Orchid and Carnation- 0.5 to 1.0 kR under *in vitro*) to obtain useful mutants. Dwivedi and Banerji (2009) irradiated the rooted stem cuttings of Dahila cv. 'Pinki' to gamma radiation at 0, 250, 500, 1000 and 1500 rads. LD_{50} on survival basis was determined in between 1000 and 1500 rads. Tiwari *et al.,* (2010) studied the effects of gamma rays in the corms of gladiolus. Increased survival percentage was reported in lower dose treated corms (5 kR). Higher the concentration *viz.,* 10 and 15 kR has reduced the survival percentage.

Thinh *et al.,* (2011) irradiated the *Phalaenopsis* orchid with 0, 20, 40, 60, 100 Gy doses with dose rate of 90 Gy/h. Three

years after irradiation treatment, there was a significant influence on growth, mutation frequencies in morphological and physiological traits of *Phalaenopsis* varieties. Survival rate and growth vigor of treated plants were with higher doses. The useful variation was the highest in 40 Gy followed by 20 Gy radiation treatments. LD_{50} dose for Phalaenopsis orchid variety is 40 Gy. Canna (*Canna generalis* L. H. Bailey) seed and rhizome were irradiated by gamma ray with five doses and found that the suitable radiation dosage for seed was 40-60 Gy and for rhizome, 10-20 Gy. The rate of germination, the rate of seedling growth and rate of rhizome survival was decreased and the mutation was raised with the increment of irradiative dosage. When the irradiative dose was over 60 Gy, the mutation rate did not vary and the plant was heavily damaged without ornamental value (Fucui *et al.,* 2011). Kayalvizhi *et al.,* (2017a) reported that, in tuberose prajwal variety bulbs were treated with different doses 0.5, 1.0, 1.5, 2.0 and 2.5 kR of gamma rays. In gamma ray 1 and 1.5 kR treated plants produced chlorophyll mutants such as Striata and also broad leaf mutants observed in 1.5 gamma ray dosage. Gamma ray 1 and 2 kR treated plants produced branched spikes in tuberose. In India, National Botanical Research Institute, Lucknow is the pioneer institution in induction of mutation in flower crops. In bougainvillea, gamma ray was used to induce various mutants and is listed in table 2 (Datta, 2014).

Kainthura and Srivastava (2015) irradiated four tuberose varieties *viz.*, Kalyani Single, Kalyani Double, Suvasini and Prajwal treated with gamma rays (0.5 kR and 1.5 kR) and X-rays (0.6 kR and 1.2 kR). The results indicated that mutagenic treatments at lower doses had significant simulative effect on some parameters *i.e.*, sprouting percentage, days taken to sprouting whereas most of the parameters showed decrease from desired parameters *i.e.*, survival rate, leaf length, number of spikes/plant, number of florets/spike, flowering duration and vase life. Higher doses of all mutagens were detrimental for vegetative and floral characters. Dhivya *et al.,* (2015) reported that crossandra (*Crossandra infundibuliformis* L.) seeds were treated with different doses of gamma rays (10, 20, 30, 40 and 50 kR). The LD_{50} value of gamma rays for seed germination and seedling survival was ranged from 20 to 30 kR. Leaf abnormalities were observed at higher doses. Stimulating effect of gamma radiation was observed at 10 and 20 kR whereas almost all the characters showed positive shift including growth and yield attributes.

Chemical mutagens

Chemical mutagenesis was first reported by Schiemann (1912) in *Aspergillus niger*. Auerbach (1967) opined that processes such as repair, transcription, translation and competitive cell growth acted as sieves for the expression of mutation and by manipulation of physical, cellular or genetic environment, frequency and spectrum of mutation could be influenced. Mackey (1967) reported that alkylating agents had a reaction pattern more suited than ionizing radiations for breaking down the buffering characteristics of polyploidy germplasm and for creating a maximum genetic diversification and allelic interaction between homologous loci. The chemical mutagens were more dependant on genetic constitution of the plant than the ionizing radiations. Chemical mutagens such as Ethylamine, Ethyl Methane Sulphonate (EMS), Methyl ethane sulphonate (MES), N-Nitrose Methyl Urea (NMU), Colchicine and Sodium Azide have been normally used (Wen and Qu, 1996). Datta (1990) reviewed that mutagenic chemicals viz., Ethyl Methane Sulphonate, Ethyl Methane Sulphonate,

Diethyl Sulphate, Ethylamine and N-Nitroso-N-Methyl urethane had been successfully used for evolving new mutant cultivars.

Effect of chemical mutagenesis in flower crops

Singh (1976) noticed a partially pollen sterile plant in petunia by treating the hybrid with 0.2 per cent EMS solution. Hentrich and Glawe (1982) found numerous mutagenic effects on shoots of carnation arising from treated axils with EMS at 1.0 or 2.5 % concentration. Fifteen new variants with altered flower colour and size were selected from the treated population. Schiva *et al.*, (1984) observed that ethyl methyl sulphonate (EMS) was more effective than sodium azide in inducing single gene mutations in gerbera. The seeds were in the mutagen solutions (6,12 or 24 h) decreased germination, growth survival, frequency of flowering plants and seed setting and no. of seeds in M_1 generation. With EMS at a low concentration and a short treatment time, the percentage of lethal mutants was reduced and a large non - lethal M_2 population was obtained.

Van Harten (1998) observed that EMS, a chemical mutagen caused high frequency of gene mutations with low frequency of chromosomal aberrations and hence it was widely used in crop plants. It was reported that genes near the centromere were more prone to mutagenic treatment than those located farther away. Chlorophyll mutants were frequent in EMS treatment but were rare in treatments with physical mutagen and this was attributed to differences in the chemical composition of the chromosomes near the centromere, making them more sensitive to chemical mutagens (Chopra, 2005). Roychowdhury and Tah (2011) treated the seeds of *Dianthus carophyllus* L. with ethyl methane sulphonate (EMS) and sodium azide (SA) at three different concentrations viz., 0.1%, 0.4% and 0.7%. It was reported that increase in the dose of EMS and SA, germination percentage and survivability were decreased at seedling stage, but they were not survived till maturity. Higher lethality over control was shown at 0.7% EMS. Pollen sterility also increased with increase in mutagenic doses. Singh *et al.*, (2013) reported that, three tuberose (*Polianthes tuberosa* L.) varieties (Calcutta Double, Prajwal and Shringar) were treated with different concentrations of EMS 0.25 % and 0.5 % for inducing mutations in qualitative and quantitative characters. EMS (0.25 % and 0.5 %) treated Prajwal variety produced seven, five and eight tepal florets than control (six tepal). In Shringar variety, 0.25% EMS concentration showed eight tepal florets in M_1 generation. Bougainvillea is a flowering plant, highly amenable for mutation induction. the various mutants induced by chemical mutagen at NBRI, Lucknow is presented in table -3.

Dhivya *et al.*, (2015) reported that crossandra (*Crossandra infundibuliformis* (L.) NEES) seeds treated with different concentrations of ethyl methane sulphonate (20, 30, 40, 50 and 60 mM) and recorded that the higher internodal length, no. of leaves, length and breadth of leaf, no. of branches and length of branch over control were observed at 30 mM EMS treated seeds. Kayalvizhi *et al.*, (2016b) reported that, tuberose variety Prajwal bulbs treated with chemical mutagens *viz.*, Diethyl Sulphate (DES) @ 15, 20, 25 and 30 mM and Ethyl Methane Sulphonate (EMS) @ 30, 45, 60 and 75 mM. The lower doses of the mutagens had recorded higher values for morphological and floral parameters than untreated control. Kayalvizhi *et al.*, (2017b) revealed that the tuberose Prajwal variety bulbs were treated with chemical mutagen DES at different doses. Nine tepal florets were observed in 30 mM of DES and eleven tepal floret was observed in 15 mM of DES.

Table.1 Gamma ray dosages adopted for different flower crops

Ornamentals	Propagation	Mutagen	Dose
Amaryllis	Bulb	Gamma rays	250 rads – 5 Krad
Bougainvillea	Stem cuttings	Gamma rays	250 rad to 1.25 kR
Canna	Rhizome	Gamma rays	2 and 4 Krad
Chrysanthemum	Rooted cuttings / suckers	Gamma rays	1 to 3.5 Krad
Gerbera	Rooted plantlet	Gamma rays	1 and 2 Krad
Gladiolus	Bulb	Gamma rays	250 rads to 5 Krad
Hibiscus	Stem cutting	Gamma rays	1 to 4 Krad
Narcissus tazetta	Bulb	Gamma rays	0.25, 0.50 and 0.75 kR
Perennial portulaca	Stem cutting	Gamma rays	250 rad to 1.25 Krad
Polianthes tuberosa	Bulb	Gamma rays	250 rad to 8 Krad
Rose	Stem with budding eyes	Gamma rays	2 to 6 Krad
Tagetes erecta	Rooted cuttings	Gamma rays	500 rad to 2 Krad
Lantana depressa	Stem cutting	Gamma rays	1 to 4 Krad

Table.2 Bougainvillea mutants induced by physical mutagen

Original variety	Characters	Mutant name	Mutagen	Characters
Partha (Single bracted)	Green leaves, bract pinkish purple, non-persistent	Arjuna	Gamma rays	Variegated leaves, creamish white, dark and light green, bract pinkish purple, non persistant
Los Banos Beauty (Double bracted)	Green leaves, persistent bract, mallow purple colour	Los Banos Variegata	Gamma rays	Variegated leaves, margin creamish white, centre green and light green, bract persistent
		Los Banos Variegata Silver Margin	Gamma rays	Variegated leaves, margin silver white, bract persistent, bract colour mallow purple
Mahara (Double bracted)	Green leaves, bract persistent, rhodamine purple colour bract	Mahara Variegata	Gamma rays	Variegated leaves, margin creamish yellow and centre green, persistent bract, rhodamine purple colour bract
		Mahara variegate Abnormal leaves	Gamma rays	Leaves are abnormal with asymmetrical lamina, light and dark green patches, margin undulated, bract persistent, rhodamine purple colour bract
Roseville's Delight (Double bracted)	Green leaves, persistent bract, burnt orange bract colour	Pallavi	Gamma rays	Variegated leaves, variegation is prominent in juvenile shoot and foliage, persistent bract, bract colour burnt orange

Table.3 Bougainvillea mutants induced by chemical mutagen

Original variety	Characters	Mutant name	Mutagen	Characters
Los Banos Beauty (Double bracted)	Green leaves, persistent bract, mallow purple colour	Los Basnos Variegata Jayanthi	0.02% EMS	Variegated leaves, centre green and light green and margin creamish yellow and white, bract persistent
Pixie (Single bracted)	Green leaves, bract pinkish purple, non-persistent	Pixie Variegata	0.02% EMS	Variegated small leaves, centre green margin creamish white and light yellow, bract pinkish purple and non-persistent

In tuberose, variety Prajwal bulbs were treated with different dose of chemical mutagens DES and EMS. The study concluded that, the highest number of florets per plant (180.00 nos.) was observed in 25 mM DES whereas minimum (40.00 nos.) was in 75 mM EMS compared to control (140.32) in M_1V_2 generation. The maximum weight of single floret (1.61 g) was noticed in 20 mM DES while minimum (1.06 g) was in 2.5 kR as compared to the control (1.15 g). (Kayalvizhi *et al.*, 2017a).

Dhawani *et al.*, 2018 reported that, the gladiolus 'Psittacinus Hybrid' corms were treated with different doses of gamma rays viz. 5.5 kR, 6.0 kR, 6.5 kR, 7.0 kR, 7.5 kR and 8.0 kR and different concentrations of DES 0.1%, 0.2%, 0.3%, 0.4% and 0.5%. It was found that DES had more pronounced effect on vegetative characters similarly for flowering characters DES produced larger spikes and with more no. of florets as compared to Gamma rays. Plants were more responsive to treatment of DES as compared to gamma rays.

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How to cite this article:

Kayalvizhi. K., A. Ramesh Kumar, A. Sankari and Anand. M. 2020. Induction of Mutation in Flower Crops - A Review. *Int.J.Curr.Microbiol.App.Sci.* 9(06): 1320-1329.
doi: https://doi.org/10.20546/ijcmas.2020.906.164